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Effects of Cephalosporium gramineum on water relations and stomatal activity in wheat

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Patricia J. Creatura

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EFFECTS OF *CEPHALOSPORIUM GRAMINEUM* ON WATER RELATIONS AND STOMATAL ACTIVITY OF WHEAT

By

Patricia J. Creatura

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ABSTRACT

EFFECTS OF *CEPHALOSPORIUM GRAMINEUM*
ON WATER RELATIONS
AND STOMATAL ACTIVITY IN WHEAT

By

Patricia J. Creatura

The stomates of wheat plants infected by *Cephalosporium gramineum* were open wider than those of healthy plants especially under conditions of water stress before the appearance of chlorotic stripes. Roots of inoculated and control hydroponically-grown seedlings were placed in polyethylene glycol solutions to produce water stress and to manipulate leaf water potentials.

The stomates in diseased plants were open wider and responded more slowly to increasing water stress in comparison to the stomatal responses of healthy plants. Diffusive resistances for both inoculated and control leaves increased as water stress was increased, indicating that the release of epidermal back pressure did not cause the significantly lower diffusion resistances of the inoculated leaves.

The diffusive resistances of inoculated leaves were lower than those of control leaves over any given range of leaf water potential(s). This alteration in the relationship between water potential and diffusive resistance of inoculated plants when compared with that of control plants implies the involvement in the disease process of a diffusible toxin, produced by the pathogen in the xylem. The differences in stomatal activity were not caused by differences in leaf water status, indicating that blockage of the vascular system by the fungus or its metabolites probably does not play a role in early pathogenesis. Graminin A, a toxin produced by C. gramineum, kindly provided by Kobayashi and Ui, caused abnormal opening of stomates in plants subjected to water stress. This implies that Graminin A has a role in disease development.

This thesis is dedicated to my husband, Bruce T. Marshall, whose continuing love, support, understanding and endurance made this thesis possible.

ACKNOWLEDGMENTS

I would sincerely like to thank Dr. Gene R. Safir and Dr. Robert P. Scheffer for making this thesis possible. Dr. Harry H. Murakishi provided helpful suggestions and assisted in the preparation of this manuscript. I am grateful to Thomas D. Sharkey of the MSU/DOE Plant Research Lab for assistance in using the gas analyzer, and to Dr. K. Kobayashi for the samples of Graminin A. I would especially like to thank Nicholas Bolgiano for preparing the figures, and Charles E. Nelsen for helpful discussion. I am grateful to many friends in the Department of Botany and Plant Pathology who also helped along the way.

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INTRODUCTION

Cephalosporium stripe disease, caused by Cephalosporium gramineum, Nisikado & Ikata, is a problem in the wheat growing areas of the world (49). There have been some studies of the ecology of the organism (29,51), of pathogenesis (24,34,42,54), and of disease control (49,51). However, these controls have not been adequate and the mechanisms of pathogenesis are still unknown.

One purpose of this thesis was to determine the pathogen-produced changes in the physiology of wheat plants by comparing the water potentials and stomatal apertures of healthy and diseased plants. The water potential versus stomatal aperture relationship was examined to determine whether or not symptoms were caused by a toxin or by reduced supply of water to the plant tissue.

The second objective originally was to determine whether or not the pathogen produced a metabolite in culture that might play a role in pathogenesis or contribute to the extent of damage caused by the disease. During the course of this study, a toxin, Graminin A, was described by Kobayashi and Ui (23) and was postulated to be a causal factor in disease development. Consequently, the second

objective was to determine whether or not Graminin A produced the same effect on the water potential versus stomatal aperture relationship of treated plants as was observed in diseased plants.

LITERATURE REVIEW

Cephalosporium stripe, a disease of wheat caused by the soil-born pathogen Cephalosporium gramineum, has been reported in wheat growing regions around the world, including Great Britain, North America, and Japan, where it was first reported in 1934 (33). Several major cereal crops are susceptible (6), as are plant species in approximately twenty genera, including grasses in the genera Bromus, Dactylis, and Poa. Spring cultivars of wheat, oats, barley, rye and triticale are susceptible, but they do not suffer serious damage from the disease in the field (49). It is thought that most infections occur at root injuries that occur during winter. Such injuries are avoided by spring planting (49). Winter wheat, the major crop affected can suffer up to 50% reductions in yield because of reduced seed set and development (49).

Cultural methods have been investigated and recommended for disease control, because of the relatively low per acre cash value of the crop. C. gramineum, which has no resistant spore form, survives between planting seasons by colonization and exploitation of residual infested host tissue (7). It has been postulated that this colonization is assisted by the production of antibiotics which prevent takeover of the infested straw by other organisms (8,9,25).

Deep plowing to cover post-harvest crop residues has been shown to reduce yield losses and disease incidence under conditions that favor rapid decay of infested plant tissues (51). Other control measures include rotation with nonhost crops, substitution of spring planted grain cultivars where possible, control of grassy weeds and delayed autumn planting (49).

Pathogenesis has been examined by several researchers. The most prominent symptom of the disease is the characteristic chlorotic stripes which form adjacent to the infected vascular bundles, usually one or two to a leaf. The stripes include adjacent interveinal parenchyma tissue and sometimes extend to include non-infected vascular bundles (48). The pathogen itself was originally observed in the xylem (29) and years later was shown to remain in the xylem while the plant was alive (48).

The hypothesis that the fungus in the xylem produces a toxic metabolite involved in the formation of the stripes led Ikata and Kawai (21) to examine culture filtrates for a metabolite active in the disease process. Filtrates obtained from cultures of C. gramineum grown in Knop's solution inhibited the growth of both roots and shoots of germinating wheat seedlings, whereas the filtrates of cultures grown on potato infusions supplemented with glucose caused stripes which were narrow and later became necrotic along the xylem vessels of leaf sheaths.

The discoloration of xylem and phloem in striped wheat stems, which occurred well above heavily invaded portions of xylem, caused Bruehl (6) to postulate the involvement of a toxin. Spalding et al

(37) examined both diseased plants and the metabolites produced in culture filtrates for clues to the cause of disease symptoms. In the soft dough stage infected plants were found to contain about 10% less moisture in the culm and head than in the lower two-thirds of the plant; moisture content in healthy plants was uniform overall. As maturity approached, the loss in moisture content was faster and greater in diseased plants than in healthy ones. The starch content of the grain was reduced, resulting in a ratio of protein to carbohydrate that was higher than normal, a characteristic associated with the kernels of wheat plants produced under dryland conditions.

When leaves in various stages of stripe development were placed in 1% Eosin Y dye to locate obstruction in the vascular pathway, striped areas failed to take up dye. Also, the lateral movement of dye in the leaf was blocked (37).

The fungus produced extracellular polygalacturonase in vivo and in vitro (39). Extracellular pectinesterases, cellulases and polysaccharides were produced in culture, but protopectinase was not evident (39). Spalding et al (39) concluded that at least three materials could block the flow of water and nutrients in the xylem. Blocking materials included conidia, hyphae, pectin plugs (formed by pectinolytic and celluloytic enzymes) and large extracellular polysaccharides. Blockage of the xylem was postulated to be responsible for at least part of the symptoms.

Wiese (48) examined young wheat plants from time of inoculation through disease development.Conidia and hyphal fragments were found in xylem vessels at the time that striping of leaves was first

evident. Occlusion of the longitudinal vessels, as visualized by acid fuchsin dye, did not occur until the vessels were filled with conidia and stripes were well developed. Plugs of polysaccharide and pectin, as observed by Spalding et al (37), were not seen during the first four weeks after inoculation. The lateral transport of dye from main vessels into the smaller lateral vessels and parenchyma tissue was found to be disturbed prior to stripe development. Wiese (48) postulated that a fungal metabolite, diffusing from the xylem, was involved in stripe development and that lateral transport was apparently affected before stripes were evident.

Other workers confirmed the production of polysaccharides (34) by C. gramineum and reported the existence of still other interesting, biologically active metabolites (8,30). Bruehl et al (8) obtained a "usable crude antibiotic extract", active against Penicillium spp, from culture filtrates of C. gramineum. Pool and Sharp (30) examined the culture filtrate fractions that contained the antibiotic and the polysaccharide. The antibiotic was found to inhibit the growth of five of seven species of bacteria tested. These results indicated that the metabolite was neither cephalosporin N nor one of the cephalosporin P series, but that it could be cephalosporin C or cephalosporic acid.

A fungal polysaccharide fraction was isolated from the straw of infected, but not from the straw of healthy wheat plants (30). Leaves from healthy wheat plants were allowed to take up 0.1% solutions of either glucose or polysaccharide prepared from culture filtrates, and then placed into eosin Y dye. Segments of the leaves

were then removed to force a lateral movement of the dye. Lateral movement of dye was interrupted in those leaves treated with polysaccharides but not in the leaves treated with glucose.

Kobayashi and Ui (23) determined the structure of a toxin, Graminin A, from culture filtrates of C. gramineum. Graminin A caused chlorosis and browning of leaves and vascular tissues of wheat cuttings at concentrations as low as 25 µg/ml. Non-host species such as soybean and corn did not develop significant browning symptoms when treated with four times that concentration. Graminin A inhibited the germination of fungal spores and the growth of many fungal and bacterial species. Three of the bacterial species tested were reported by Pool and Sharp (30) to be inhibited by substances produces by cultures of C. gramineum. Growth of two of those species was prevented by a concentration of less than 25 µg/ml of Graminin A. Growth of the third species was not inhibited until a concentration greater than 200 µg/ml was used. Of the fungal species tested, Trichoderma viride, an aggressive antagonist of C. gramineum (24), required the highest concentration (195 µg/ml) of Graminin A to cause 50% inhibition of spore germination.

These authors all propose that a metabolite, either an extracellular polysaccharide or a smaller molecule that acts as a toxin or is an antibiotic, is produced by the pathogen while in the xylem and is a primary agent in pathogenesis. The molecule, if a polysaccharide, could be carried passively by water moving from the xylem where it could presumably plug the small lateral vessels or the pit membranes in the walls of the xylem vessels (20). This would

decrease the flow of water and other xylem-transported nutrients from the xylem to surrounding parenchyma tissue. Alternatively, a toxin or an antibiotic active against plants could diffuse out of the xylem and affect the plasmalemma or some other cellular organelle, disrupting the normal functions of the parenchyma cells and thus causing chlorosis.

Many pathogens disrupt the uptake, transport, or loss of water from plants. Wilted leaves, flaccid stems, desiccated necrotic areas, and water-soaked lesions are examples of obvious disruption. Many efforts have been made to determine where the disruption occurs in diseases other than Cephalosporium stripe. Some of this work will be reviewed briefly, although there are several comprehensive reviews (4,14,39).

Toxins are known primary agents in development of Victoria blight of oats, milo disease of sorghum, and maize leaf blight (34). The cells of host plants treated with these toxins have been shown to leak electrolytes (34), indicating that normal permeability of the cellular membranes was altered.

Fusicoccum amygdali and its toxin, fusicoccin, cause a severe wilt of peach and almond shoots. Fusicoccin increases stomatal opening both in the light and in darkness (45), thereby increasing transpiration to a level at which the leaves wilt because the affected plant transpires water faster than the xylem can conduct. The increase in stomatal opening was caused by increased permeability in the plasindemma of guard cells, resulting in increased K^+ uptake (41,43). Consequently, the stomatal apertures

of treated leaves were found to be greater and water potentials far lower than those of healthy leaves (18,44). In contrast to fusicoccin, the toxins of Helminthosporium victoriae (42) and H. maydis race T (1), both of which cause leakage of electrolytes by the plasmalemmae, have been shown to close stomates in light.

It is possible to determine whether the cause of abnormal stomatal action was blockage of the vascular system or a diffusible toxin by examining the relationship between water potential and stomatal aperture. The basis for such a differentiation was first described by Duniway (15,16) and stated succintly by Ayres in his review:

> If the sole factor causing stress in leaves is a reduced supply of water to the tissue then the relationship between stomatal diffusion resistance and either relative water content, or leaf water potential, which is characteristic of each species, will be unaltered by disease (4).

If this relationship is found to be altered, then the presence of a toxin can be postulated.

One disease in which stomatal action has been studied is Fusarium wilt of tomato. The pathogen, Fusarium oxysporum f. lycopersici, causes a wilt of tomato which has been the subject of a great many papers. The point in question was whether a toxin or blockage of the xylem caused the particular symptoms of the disease. Several papers were concerned with the isolation and evaluation of toxic metabolites produced in vitro, and sometimes in vivo. Fusaric acid and lycomarasmin were discounted as primary agents in disease development because they gave symptoms that differed from those of the disease (39). Lycomarasim was also eliminated because

it was thought to be released when the pathogen lysed (11). Conductance of water was found to be less in the stems of diseased plants than in healthy ones (26). Scheffer and Walker found that the pathogen was always associated with wilt symptoms and that blockages could be detected by the use of dye. In reviewing all the known data, Scheffer and Walker concluded that the primary cause of the wilt symptom was blockage of the xylem, particularly in the petiole (35).

This conclusion was supported by Duniway (12,13) who confirmed the results of the earlier researchers both by repeating their experiments using similar procedures and by using newly available instrumentation. The resistance of the leaflet was found to be highest in the transpirational pathway. The resistance of water conductances in the petioles of infected plants was shown to be at least 400 times greater than that of the petioles of healthy plants (12). The solute potential of diseased plants was lower than that of healthy plants when the water potential of both was zero, indicating that leaf wilting was not caused by a loss of solute resulting from a change in permeability of the plasmalemma (13). The stomatal resistances of diseased plants were found to be as high or slightly higher than that of healthy plants at the same water potential (13). Since there was no change in stomatal behavior that could be responsible for wilting, it was concluded that wilting was not caused by a toxin, but by a reduced supply of water to the wilted tissue.

Other diseases have been examined to determine whether or not the relationship between potential and stomatal apertures of infected plants differs from that of healthy plants. The black root disease of sugar beet reduced transpiration and increased diffusive resistance more in susceptible cultivar than in a resistant cultivar (34). However, the relationship between stomatal aperture and water potential was not altered by the disease. Inoculated plants and water-stressed healthy plants of the susceptivle cultivar had water potentials and diffusive resistance values in the same ranges. The cause of the reduced transpiration and large stomatal diffusive resistances in inoculated plants of both cultivars was a reduced water supply to the leaves, produced by increased resistance to water transport in the plant. These findings were predicted by the absence of change in the relationship between water potentials and stomatal apertures.

Therefore, the examination of the relationship between water potentials and stomatal apertures in diseased plants has been and can be used to differentiate between abnormal stomatal activity caused by a diffusible toxin or by blockage of the vascular system.

MATERIALS AND METHODS

Plant Material

Wheat seedlings (Triticum aestivum L. cv Genesee) were grown hydroponically or in pots of steamed loam soil, approximately ten seedlings per pot. The potted plants were grown in pasteurized loam soil in a growth chamber at a constant temperature of 19°C. Sixteen hours of light were provided by fluorescent and incandescent lights (intensities 18.3×10^3 and 1.1 lumens/m^2 respectively).

For hydroponic culture, seeds were germinated on wet Whatman #1 filter paper in petri dishes. Seedlings with radicles 1 cm long were placed on galvanized screen mesh racks (15-20 seedlings per rack), suspended over nutrient solutions in trays. They were grown under Sylvania Gro-Lux bulbs at room temperature, which was about 22°C. Seven days after seeding, the roots of seedlings were placed in five liter beakers, one rack per beaker, which were filled with a modified Hoagland's solution, diluted 1:1 with distilled water (Table 1) (47). The plants in beakers were held in a growth chamber with the same day length, light intensities, and temperature used for the potted plants.

The first (oldest) leaves of control and experimental seedlings were inoculated on day 10. Aerators were placed in the beakers on

TABLE 1. Preparation of modified Hoagland's solution, diluted 1:1 with water.

Nutrient	Stock Solution (g/l)
Solution A	
Calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$	295.0
Sequestrene 13% NaFe*	38.44
Solution B	
Potassium phosphate KH_2PO_4	34.25
Potassium nitrate KNO_3	126.65
Magnesium sulphate $MgSO_4 \cdot 7H_2O$	62.50
Zinc sulphate $ZnSO_4 \cdot 7H_2O$	0.056
Manganous sulphate $MnSO_4 \cdot H_2O$	0.391
Copper sulphate $CuSO_4 \cdot 5H_2O$	0.021
Boric acid H_3BO_3	0.725
Molybdic acid $MoO_3 \cdot 2H_2O$	0.005

1. Calcium nitrate and sequestrene were combined in stock solution "A". All other salts were in solution "B".

2. Both stock solutions "A" and "B" were diluted 1:500 with water for growing plants.

*Ciba-Gregy, New York.

day 11, and on day 16 the beakers were cleaned and refilled with fresh Hoagland's solution. Water potentials and diffusive resistances were measured when the plants were 18 days old, eight days after inoculation.

Inoculation Procedure

A virulent Michigan isolate, C1 (courtesy of A.V. Ravenscroft, Department of Botany and Plant Pathology, Michigan State University), was reisolated from inoculated wheat plants every three months. Inoculum was prepared from cultures grown for 7-12 days on potato dextrose agar. The culture surface was flooded with sterile water, and spores were dislodged by scraping. The suspension was adjusted to 10^6-10^7 spores per ml, using a hemacytometer. Plants were inoculated at two points in the culm at the base of the first or second leaf using a hypodermic syringe with a 20 gauge needle. Control seedlings were injected with water rinsed from a sterile potato dextrose agar plate.

The plants were examined for symptom development during the experiments and for 12 additional days. Leaf segments were then placed on potato dextrose agar or on Ravenscroft's green plant agar (50) and incubated to confirm the presence of the pathogen.

Diffusive Resistance Determination

A. Measurement of diffusive resistance in plants

An autoporometer (Li-cor Limited — Lambda Instruments Corporation, Lincoln, Nebraska; LI 65 Autoporometer^{TM} with an LI-20S diffusive resistance sensor equipped with a No. 20SA 3.5 x 20 mm special aperture) was used for determining diffusive resistances (22,46). Relative diffusive resistances (x 10^{-2} sec) and leaf temperatures (°C) were determined, alternating between inoculated and control seedlings, at the middle of the adaxial surface of either the first or second leaf (40). The relative diffusive resistances were first corrected for temperature differences on a standard 25°C basis, with factors supplied by the manufacturer, and then converted to diffusive resistances (sec/cm) with the regression equation derived in section B.

B. Autoporometer calibration

The autoporometer sensor was calibrated with the 201S calibration plate, using three layers of Whatman number one chromatography paper under one layer of Whatman number fifty filter paper. The chromatography paper had been cut into strips, 13 cm by 33 cm, and protruded from two opposite sides of the calibration plate. The calibration plate was placed on top of an inverted one-liter beaker for support, and each end of the chromatography paper was placed in a one-liter beaker of distilled water. These two beakers were set on opposite sides of the inverted support beaker.

The layers of chromatography paper acted as a wick for the distilled water. The calibration plate equilibrated overnight in

a plastic dishpan, lined with wet paper towels, and covered with a piece of plastic film. The sensor was attached to the calibration plate, conditioned by cycling ten times, and allowed to equilibrate for one hour. The plastic and the dishpan were then removed.

Relative diffusive resistances were determined by time necessary for the relative humidity inside the autoporometer sensor to change from 18% to 20%. Four resistance fields of the calibration plate were usable with the No. 20SA autoporometer aperture. One relative diffusive resistance ($x\ 10^{-2}$ sec) with its associated temperature measurement from each of the four resistance fields was taken in turn until ten measurements per resistance field were accumulated. The relative diffusive resistances were corrected for temperature differences. Using the mean relative diffusive resistances from the four resistance fields as the dependent variable and the manufacturer's measured resistances (sec/cm) of those fields as the independent variable, a regression equation was calculated:

$$x = \frac{y - 54.36}{20.38}$$

Water Potential Determinations

Water potentials were determined with a commercial dew point hygrometer [Wescor Inc., Logan, Utah; Model HR-33 (T)] with C-52 sample chambers (28). Readings for leaf tissue samples and PEG solutions were taken in the dewpoint mode and readings for Hoagland's solution were taken in the pychrometer mode.

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Polyethylene Glycol Solution Preparation

Solutions of polyethylene glycol 4000 (PEG) (Sigma Chemicals, molecular weight 3000-3700 daltons) of specific approximate water potentials were prepared by first dissolving weighed amounts of PEG (Table 2) in small amounts of distilled water and then bringing the solution up to the desired volume with more water. The quantity of PEG needed to prepare a solution of a desired water potential was determined empirically, using the Wescor hygrometer.

Effect of Infection on Stomatal Conductances as Determined by Gas Analysis Techniques

Hydroponically-grown seedlings were used one at a time, with the first leaf secured in a sensing chamber. One leaf each was used from one control and one inoculated plants. Stomatal conductances of both the adaxial and abaxial sides of the leaf, the evaporation rate of water out of the leaf, and the assimilation of carbon dioxide by the leaf were measured, at two-minute intervals. Standard procedures, developed by Sharkey (36), Raschke (31) and others of the MSU/DOE Plant Research Lab, were used, modified for most monocot leaves.

Gas exchange by the leaf was monitored by measuring humidification and CO_2 depletion of air passing over the 1.19 cm^2 surface area of leaf tissue present in the sensing chamber. The flow rate was 50 l/hour over the adaxial and abaxial surfaces. Changes in the molar fluxes of H_2O and CO_2 were measured with infrared gas analyzers (URAS 2, Hartmann and Braun, Frankfurt A.M., Germany).

TABLE 2. Preparation of polyethylene glycol 4000 (PEG) solutions of approximate water potentials.

Water Potentials (bars)	PEG (g/l)
-4	133.3
-6	186.7
-7	206.7
-8	230.0

The temperature of the leaf, which was monitored with a fine thermocouple pressed against the abaxial side, ranged from 22 to 26°C. The dewpoint of the air was kept constant at 18°C.

The intercellular CO_2 concentration was calculated using the following equation:

$$C_i = C_a - 1.6A\ r$$

where C_a is the CO_2 concentration of the air passing over the leaf, A is the assimilation rate, and r is the resistance to water vapor loss of the stomata and boundary layer. The factor 1.6 is the ratio of diffusivities of water and CO_2.

White light was provided by an Osram XBI 6000 W water cooled Xenon arc lamp shining through a Corning no. 4600 infrared glass filter. The light intensity was reduced by using neutral density Plexiglas filters (no. 800 and 838, Rohm and Haas, Darmstadt, Germany). The light intensity was 190 w/m^2 (570 $\mu E/m^2 sec$) measured with an Eppely pyranometer. Monochromatic light was provided by an air-cooled 2.5 kw Xenon arc lamp. Blue light was produced by interposing a 470 nm short wave pass and 370 nm long wave pass filter. After these band pass filters, the light went through a 436 nm interference filter (1/2 band width 15 nm) which was water cooled. Red light was produced by passing the light through a 850 nm short wave pass and 660 nm long wave pass filter, plus a 680 nm interference filter (1/2 band width 18 nm). To obtain 740 $\mu E\ m^{-2} sec^{-1}$, two no. 800 Plexiglas neutral density filters were used with the red light.

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µl/l of air. Low and moderate levels of water stress were produced in the plants by placing the roots of the seedling in Hoagland's solution (approximately -0.3 bars) and in PEG (approximately -7 bars), respectively.

Conductance, a measure of stomatal aperture, is the reciprocal of diffusive resistance. Total conductance are the sums of both the adaxial and abaxial conductances. Mean total conductances are the arithmetic means of four total conductances, determined successively at essentially the same internal carbon dioxide concentration.

Effects of a Fungal Toxin, Graminin A, on the Stomatal Activity of Wheat

Graminin A, produced by C. gramineum, was kindly provided by K. Kobayashi (Department of Botany, Hokkaido University, Japan). The roots of eighteen-day-old hydroponically-grown seedlings were placed in a PEG solution of approximately -6 bars for one hour. Graminin A in several concentrations (5, 15, 100, and 500 µg/ml), in 2% ethanol, was painted on the adaxial surface of each first leaf on intact seedlings. Control leaves were painted with a 2% ethanol solution which is known to be without effect on leaves (23) or stomates (36). This method is similar to that of Turner (42) and Graniti and Turner (18). Diffusive resistances were determined at 0.5 and 1.5 hours after the application of Graminin A.

RESULTS

Diffusive Resistances of Control and Inoculated Plants During Infection

The second leaves of twelve experimental seedlings and ten control seedlings were inoculated 14 days after seedling. Diffusive resistances of these leaves were measured before inoculation and on various days during disease development. The experiment was repeated twice.

Eight and ten of the plants that were inoculated actually became diseased in the first and second experiments respectively. Only the diffusive resistances of leaves that were found to be infected were included in the calculation of the mean diffusive resistances for inoculated plants.

The diffusive resistances of the inoculated plants were less than that of the control plants the first seven days after inoculation (Table 3, Figure 1). The diffusive resistances of the inoculated and control plnats were significantly different, at the 0.05 level using a T test, as early as day 1 after inoculation and also on days 2, 3, 5 and 6, in the first experiment. From day 7 to day 13, the mean diffusive resistances of inoculated and healthy plants were not significantly different. On days 7, 11 and 13, the

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TABLE 3. Effect of infection on the adaxial stomatal diffusive resistances of leaves. Mean values (sec/cm) of the second leaves are given.

Day	Control	Inoculated	Symptoms
0	13.14	11.93	
1*	13.60	9.61	
2*	11.65	9.22	
3*	14.77	10.29	
4	12.72	11.10	
5*	12.55	8.21	
6*	13.65	11.28	
7	12.42	10.90	
9	13.56	14.42	
11	18.15	14.39	
12	19.07	19.52	Distinct chlorotic stripes necrotic leaf sheath veins
13	21.32	21.16	
14*	21.80	36.00	leaves chlorotic overall
16*	18.07	52.45	necrotic regions on leaf blades

*The mean diffusive resistances of control and inoculated plants for that day are statistically different at the 0.05 level using a T test.

FIGURE 1. Effect of infection on the adaxial stomatal diffusive resistances (sec/cm) of the second leaves.

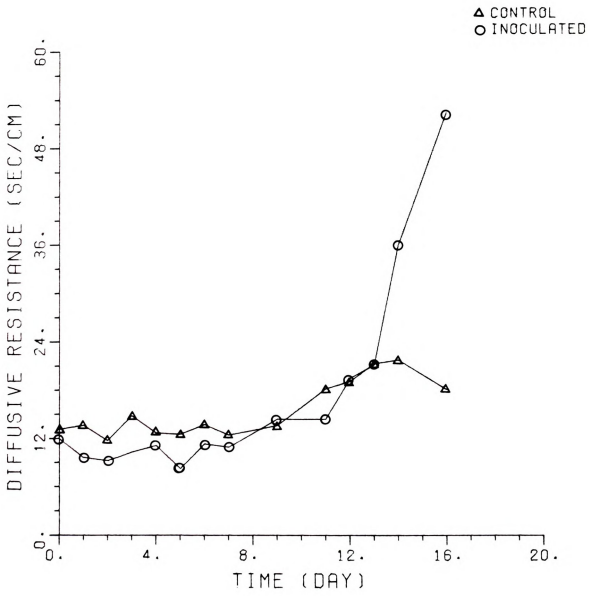
△ CONTROL
○ INOCULATED
DIFFUSIVE RESISTANCE (SEC/CM)
0.
12.
24.
36.
48.
60.
0.
4.
8.
12.
16.
20.
TIME (DAY)

Figure 1

mean diffusive resistances of the inoculated leaves were smaller than that of the control leaves, while on days 9 and 12, the opposite was true. During this time the mean diffusive resistances for the control leaves increased from 12.4 and 13.6 sec/cm on days 7 and 9, respectively, to 19.1 and 21.3 sec/cm on days 12 and 13 as the leaves aged.

The mean diffusive resistance of the inoculated leaves increased from 10.9 and 14.4 sec/cm on days 7 and 9, respectively, to 19.5 and 21.2 on days 12 and 13. Part of the increase in the mean diffusive resistances of the inoculated leaves was also correlated with aging of the leaves. But a substantial amount of this observed increase in the mean diffusive resistances resulted from infection. By day 12, distinct chlorotic stripes were present on all of the eight inoculated plants. The leaf sheath veins were necrotic in six of these eight plants. Areas of the inoculated leaves that were not part of the stripes became chlorotic by day 14, and on day 16 regions of the inoculated leaves were necrotic. The large mean diffusive resistances of 36.0 and 52.5 sec/cm for the inoculated leaves on days 14 and 16, respectively, indicated that the stomates were closed in the dying leaves. These mean values also were significantly greater than the mean diffusive resistances of the control leaves, which were 21.8 and 18.1 sec/cm on days 14 and 16, respectively.

The very large diffusive resistances, indicating stomatal closure which occurred after symptoms developed probably were related to death of the leaves and thus were not of interest.

Smaller, but significantly different mean diffusive resistances occurred early in infection and before chlorotic stripes appeared

Diffusive Resistance of Infected and Control Leaves Under Moisture Stress

Diffusive resistances of the first leaves of ten control and twelve inoculated hydroponically-grown seedlings were measured while the roots were still in Hoagland's solution (approximately -0.3 bars) and under little water stress. The means of the diffusive resistances of inoculated and control plants were not statistically different at the 0.05 level, using a T test (Table 4, Figure 2).

The roots of seedlings then were held for one hour in a -4 bar PEG solution (27). Diffusive resistance readings were taken as before, after which the seedlings were placed into a -6 bar PEG solution. If necessary, the procedure was repeated using -7 and -8 bar solutions to expose the plants to sufficient moisture stress, for comparison of stomatal behavior in infected and control plants. The water stress on seedlings produced by the PEG caused the diffusive resistances for both inoculated and control plants to increase greatly (Table 4, Figure 2), indicating that the stomates became increasingly more closed. The mean diffusive resistances of the inoculated plants were smaller (i.e., stomates were more open) than the mean diffusive resistances of the control seedlings. One exception occurred in the second experiment, in which the mean diffusive resistance of the control (14.24 sec/cm) was less than that of the inoculated mean (16.07 sec/cm). In each of the four

TABLE 4. Effect of infection on diffusive resistances of water-stressed infected and control leaves. Water stress was induced by a series of Hoagland's and PEG solutions of increasingly negative water potentials (bars). The mean adaxial stomatal diffusive resistances (sec/cm) of the first leaves are given.

Experiment	Plants	Water Potentials of Solutions				
		Hoagland's Solution -0.3	PEG -4	PEG -6	PEG -7	PEG -8
1	Inoculated	8.09	13.11*	39.53		
	Control	7.95	21.88*	54.23		
2	Inoculated	10.14	16.07	19.26	26.35	50.55*
	Control	8.63	14.24	34.05	44.59	94.83*
3	Inoculated	6.81	10.21*	14.23*	31.02	
	Control	9.45	28.00*	38.26*	65.06	
4.	Inoculated	6.67	11.58	17.18	27.13	40.87*
	Control	6.21	14.13	20.21	49.04	60.18*

*Pairs of mean diffusive resistances that are statistically different, at the 0.05 level, using a T test.

FIGURE 2. Effect of infection on diffusive resistances of leaves subjected to moisture stress. Roots of seedlings were placed in a series of Hoagland's (approximately -0.3 bars water potential) and PEG (approximately -4, -6, -7, -8 bars) solutions were used to induce moisture stress. Adaxial stomatal diffusive resistances (sec/cm) from experiment 4 (Table 4) are presented.

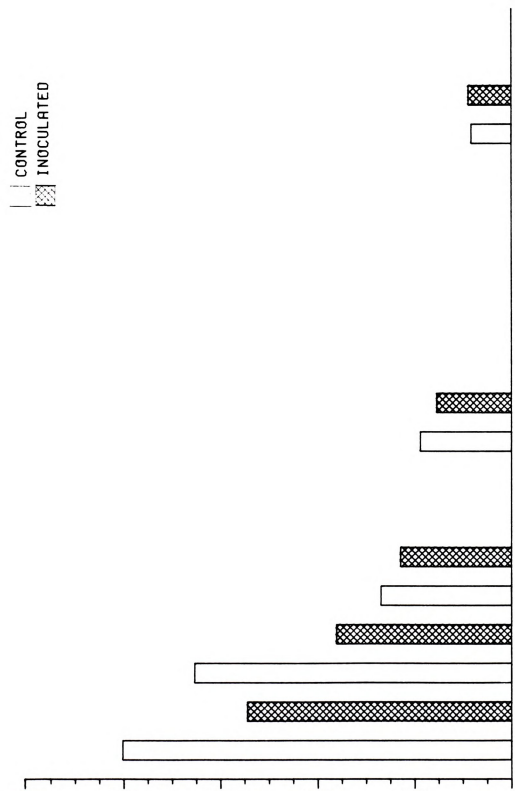
CONTROL
INOCULATED
DIFFUSIVE RESISTANCE (CM/SEC)
0.
15.
30.
45.
60.
75.
-8
-7
-6
-4
-0.3
WATER POTENTIAL (BARS)

Figure 2

experiments during exposure to at least one PEG solution of the various water potentials used, the mean diffusive resistance of the inoculated plants was statistically smaller than that of the control plants when a T test was used at the 0.05 level. In experiment 4, for example, there were no significant differences between infected and control seedlings growing in Hoagland's solution or in PEG solutions at -4, -6, or -7 bars. However, the infected and control seedlings differed significantly when placed in the PEG solution of -8 bars. The mean diffusive resistance of the inoculated plants was 40.87 sec/cm which is significantly smaller than 60.18 sec/cm, the value recorded for the control seedlings.

These data show that stomates of inoculated plants were more open than those of control plants, when plants were placed under water stress. However, stomates of both inoculated and control plants responded to the series of increasingly more negative water potentials with increasingly greater diffusive resistances. This suggests that the ability to respond to water stress by stomatal closure was not destroyed. Rather, the stomatal response of diseased plants seemed to be less efficient or less complete than that of healthy plants.

Effect of Infection on Water Potentials and Diffusive Resistances in Plants Under Moderate Moisture Stress

The first leaves of hydroponically-grown, 18-day-old seedlings were washed twice, using glass distilled water and cheesecloth, to remove salts resulting from splashing of Hoagland's solution by

aeration. The roots of seedlings were placed in a PEG solution with a water potential of approximately -7 bars. Diffusive resistances were checked periodically, using the autoporometer, until the leaves showed signs of stress. The diffusive resistance of a leaf was measured and the leaf was excised, at the edge of the autoporometer sensor proximal to the plant, and placed on a template marked to facilitate excision of the sample. A 13 mm slice of the area measured by the autoporometer sensor was quickly excised from the leaf by means of two parallel cuts perpendicular to the length of the leaf. Forceps were used to place the leaf segment in the sample holder. The sample holder was inserted into the sample chamber, which was then immediately sealed. This procedure was repeated, alternating between inoculated and control leaves, until all the sample chambers were sealed. Readings for each chamber were recorded every hour and final readings were recorded after a standardized equilibration time of four hours. The procedures are based on those suggested by Nelsen et al (28).

This experiment was repeated three times, using varying numbers of samples (6-12) of control and inoculated leaves at different times of day. Using water potential as the independent x variable and relative diffusive resistance as the dependent y variable, a data point for each leaf sampled was plotted (Figure 3). The mean water potential values of the inoculated and healthy leaves, -9.00 and -9.28 bars, respectively (Table 5), did not differe statistically at the 0.05 level using a T test. The mean diffusive resistance values of the inoculated leaves was 17.42 sec/cm, which was smaller

TABLE 5. Effects of infection on water potentials and diffusive resistances of leaves. Values are the water potentials (bars) and adaxial stomatal diffusive resistances (sec/cm) for the first leaves of control and inoculated wheat plants exposed to moderate water stress induced by a PEG solution of approximately -7 bars water potential.

Experiment	Control		Inoculated	
	Water Potential (bars)	Diffusive Resistance (sec/cm)	Water Potential (bars)	Relative Diffusive Resistances (sec/cm)
I	-8.13	183.14	-14.00	16.23
	-8.27	27.28	-6.13	34.06
	-4.00	221.08	-7.33	18.40
	-8.67	27.82	-8.27	23.49
	-13.73	51.85	-7.87	24.71
	-11.30	46.73	-10.00	29.32
	-7.73	18.03	-8.67	16.35
	-14.93	20.38	-10.80	14.41
	-8.00	18.99	-7.87	15.48
	-8.40	24.28	-9.47	11.91
	-8.00	48.95		
	-6.53	45.67		
II	-10.40	7.92	-9.86	12.66
	-10.40	9.28	-9.33	17.61
	-8.27	25.61	-8.67	16.05
	-8.50	30.15	-10.67	8.71
	-10.80	15.41	-9.87	9.92
	-9.20	31.39	-6.53	12.46
	-11.20	28.65	-9.20	22.65
	-9.20	51.85	-8.30	19.06
	-12.80	66.10	-10.00	21.92
III	-10.00	56.40	-8.67	19.46
	-13.30	37.41	-11.33	19.97
	-14.00	52.74	-7.07	16.33
	-11.45	27.24	-10.67	12.10
	-18.00	91.23	-8.51	8.08
	-12.67	23.15	-5.87	4.79
Means	-9.28	47.69*	-9.00	17.42*
Variances	2.37	2355.03**	1.80	44.15**

* differ at the 0.05 level using a T test
** differ at the 0.05 level using an F test

FIGURE 3. Effect of infection on water potentials and diffusive resistances of water stressed leaves. Each point represents the water potential (bars) and adaxial stomatal diffusive resistance (sec/cm) of either a control or an infected leaf. Moderate water stress was induced by a PEG solution of approximately -7 bars water potential.

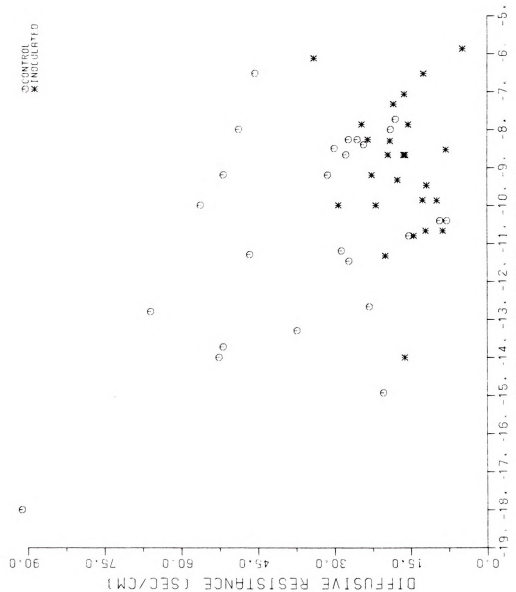
O CONTROL
* INOCULATED
DIFFUSIVE RESISTANCE (SEC/CM)
0.0
15.0
30.0
45.0
60.0
75.0
90.0
-5. -6. -7. -8. -9. -10. -11. -12. -13. -14. -15. -16. -17. -18. -19.
WATER POTENTIAL (BARS)

Figure 3

than 47.69 sec/cm, the mean for the control leaves. These means were statistically different at the 0.05 level using the T test.

These data show that the diffusive resistances of inoculated leaves were lower than those of control leaves over any given range of leaf water potential. The differences in stomatal aperture were not caused by differences in leaf water status. Therefore, the relationship between leaf water potential and stomatal diffusion resistance was altered by infection.

Effect of Infection on Stomatal Conductance as Determined by Gas Analysis

The roots of both inoculated and control plants were grown in Hoagland's solution (approximately -0.3 bars) and were consequently under little water stress. Stomatal action in both control and inoculated leaves seemed to be sensitive to the presence of light (Figures 4, 5). When the xenon light was turned on, mean total conductances of leaves from the control and inoculated plants increased greatly, indicating that the stomates had opened. Light has a direct effect on stomatal action in several plant species (36).

The internal carbon dioxide concentration of the leaf has an effect on stomatal conductance (31). Therefore carbon dioxide was increased by raising the concentration in the air supplied to the leaf by increments of 100 µl, from approximately 20 µl/l, to about 530 µl/l. This increased the internal CO_2 concentration from 17.9 to 429.75 µl/l, which caused total conductance of the control leaf to increase from 38.7 to 54.5 x 10^{-2} moles/m^2-sec (Figure 6). The total

FIGURE 4 (a,b). Effect of infection on the stomatal conductances of the first leaf of a healthy seedling subjected to varying amounts of water stress. Roots of the seedling were placed in Hoagland's (approximately -0.3 bars water potential) and PEG (approximately -7 bars) solutions to induce small and moderate amounts of water stress. Mean conductances ($\times 10^{-2}$ moles/m^2sec), determined from successive gas analysis samples, are given.

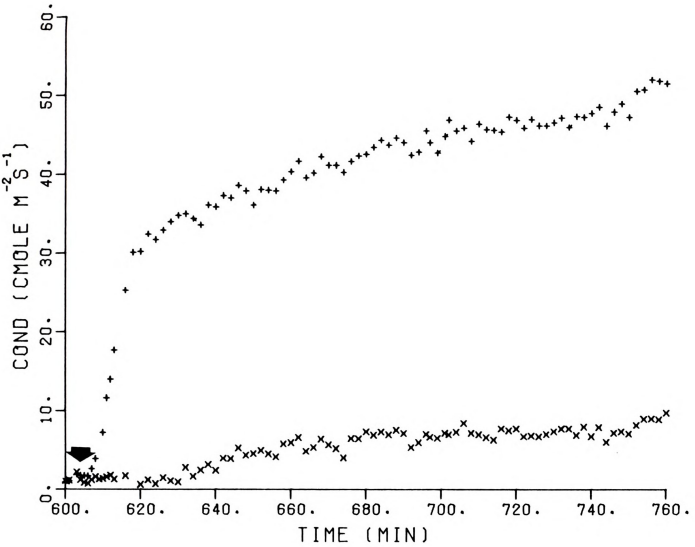

Figure 4A. Arrow indicates time the light was turned on.
+ adaxial conductance
x abaxial conductance

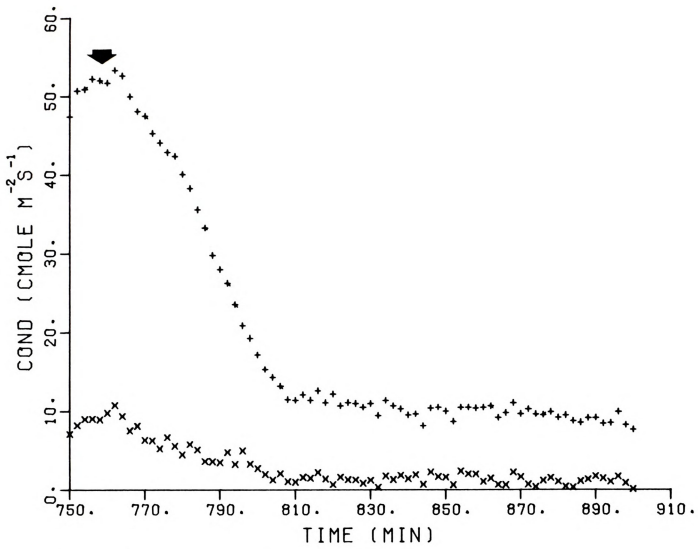

Figure 4B. Arrow indicates time seedling roots were moved from Hoagland's solution to the PEG solution.
+ adaxial conductance
x abaxial conductance

FIGURE 5 (a,b). Effect of infection on the stomatal conductances of the first leaf of an inoculated seedling subjected to varying amounts of water stress. Roots of the seedling were placed in Hoagland's (approximately -0.3 bars water potential) and PEG (approximately -7 bars) solutions to induce small and moderate amounts of water stress. Mean conductances (x 10^{-2} moles/m^2 sec), determined from successive gas analysis samples, are given.

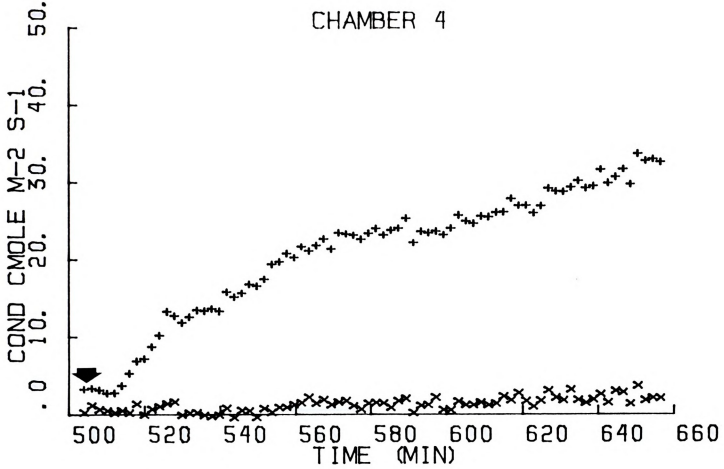

Figure 5A. Arrow indicates time the light was turned on.
+ adaxial conductance
x abaxial conductance

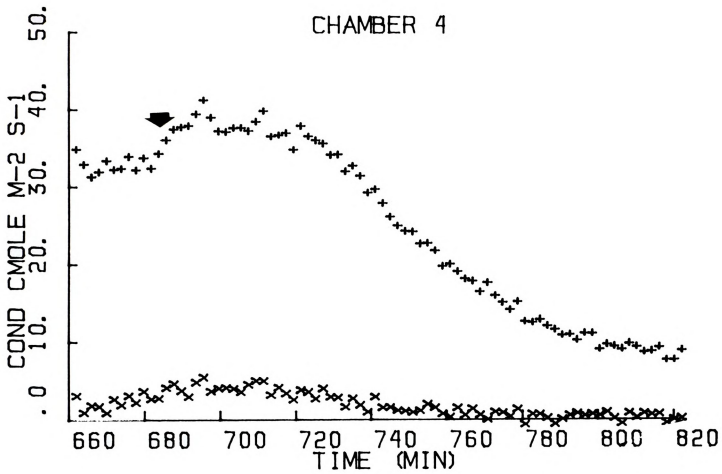

Figure 5A. Arrox indicates time seedling roots were moved from Hoagland's solution to the PEG solution.
+ adaxial conductance
x abaxial conductance

FIGURE 6. Effect of infection on carbon dioxide assimilation of water stressed leaves. Small and moderate amounts of water stress were produce by placing roots of the plants in Hoagland's (approximately -0.3 bars water potential) and PEG (approximately -7 bars) solutions, respectively. Mean assimilations (μmoles/m^2sec), determined from successive gas analysis samples, are given.

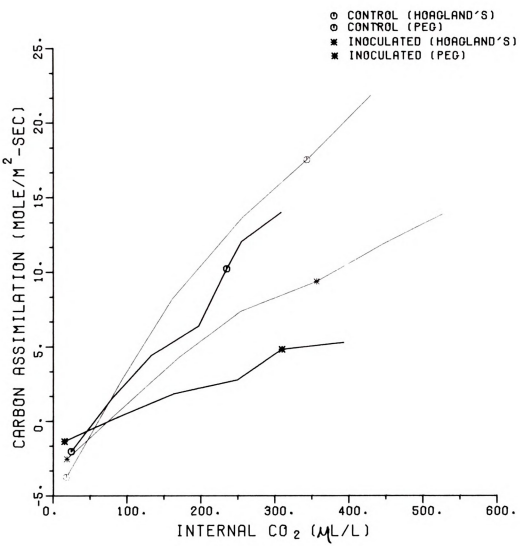
CONTROL (HOAGLAND'S)
CONTROL (PEG)
INOCULATED (HOAGLAND'S)
INOCULATED (PEG)
CARBON ASSIMILATION (MOLE/M2-SEC)
INTERNAL CO2 (µL/L)
25.
20.
15.
10.
5.
0.
-5.
0.
100.
200.
300.
400.
500.
600.

Figure 6

conductance of the inoculated leaf increased from 23.6 to only 34.68 x 10^{-2} moles/m^2-sec, when its internal CO_2 concentration was raised a comparable amount, from 18.35 to 444.75 µl/l (Table 6). Approximately 170 minutes were required for the total conductance of the inoculated leaf to accomplish that change (Figure 4) while only 145 minutes were needed for the control leaf to change (Figure 5). The conductance of the control leaf, therefore, increased more quickly and reached a greater value than the conductance of the inoculated leaf (Table 6, Figures 4, 5).

These results were typical of the data recorded during this experiment. The control and infected leaves had the same type of response (i.e., an increase in conductance in response to the experimental conditions) but the control leaf responded faster and, usually, to a greater degree than the infected leaf (Figure 4, 5). Under conditions of mild stress, the peak total conductance for the control leaf was greater than that of the infected leaf (54.5 and 34.68 x 10^{-2} moles/m^2-sec, respectively). Also, the peak adaxial and abaxial conductances of the healthy leaf (47.225 and 7.32 x 10^{-2} moles/m^2-sec, respectively) were greater than those of the infected leaf (33.075 and 2.915 x 10^{-2} moles/m^2-sec, respectively), under mild water stress. However, the adaxial conductances were greater than the abaxial conductances for both control and inoculated leaves (Table 6, Figures 4, 5).

When infected and control plants were stressed by placing their roots in the PEG solution, the adaxial and abaxial conductances decreased for both leaves (Figures 4, 5). The total conductances

TABLE 6. Effect of infection on the stomatal conductance and carbon dioxide assimilation of leaves under little and moderate water stress. Seedling roots were placed in Hoagland's solution (approximately -0.3 bars water potential) and PEG (approximately -7 bars), to produce small and moderate amounts of water stress, respectively.*

Solution	Control			Inoculated		
	Internal $[CO_2]$[a]	Conductance[b]	Assimilation[c]	Internal $[CO_2]$[a]	Conductance[b]	Assimilation[c]
Hoagland's Solution (approximately -0.3 bars)	17.90	38.70	-3.75	18.35	23.60	-2.35
	93.80	45.40	2.84	87.43	25.15	0.61
	161.75	49.33	8.24	170.25	26.03	4.28
	256.50	51.20	13.68	254.00	29.20	7.37
	343.75	53.70	17.55	356.75	33.47	9.37
	429.75	54.50	21.85	444.75	34.68	11.85
				530.75	35.98	14.95
PEG (approximately -7 bars)	24.20	13.43	-2.04	14.93	11.98	-1.37
	79.03	11.76	1.50	87.40	9.68	0.26
	133.00	11.75	4.43	162.50	9.13	1.81
	197.25	10.78	6.38			
	235.00	10.08	10.22			
	255.25	8.04	12.06	249.50	10.32	2.76
	308.50	7.74	14.00	309.75	9.03	4.82
				393.00	8.18	5.27

[a] $\mu l/l$; [b] x 10^{-2} moles/m^2-sec; [c] μmoles/m^2-sec

*Mean conductances and assimilations, determined from successive gas analysis samples, are given.

were decreased and finally reached a lower, more narrow range of values (from 7.7 to 13.43 x 10^{-2} moles/m^2-sec) which held steady until the experiment was terminated (Table 6). Infected and control leaves reached essentially the same final total and adaxial conductance values, but the control leaf took approximately 60 minutes (Figure 4) and the inoculated leaf took 105 minutes to reach the final values (Figure 5). Therefore, the control leaf reacted much more quickly than the inoculated leaf, in which the stomates were held on substantially longer.

Gas analysis showed that total carbon dioxide assimilation rate was much lower for the inoculated leaf than for the control leaf (Table 6, Figure 6). When the plants were in Hoagland's solution and the internal carbon dioxide concentration for both leaves was approximately equal, the carbon dioxide assimilation rate of the inoculated leaf was 11.85 µmoles/m^{-2}-sec. In contrast, the assimilation rate of 21.85 for the control leaf when the internal CO_2 concentration was approximately 430 µl/l for each (Table 6, Figure 6). Under moderate water stress induced by PEG, the assimilation rates for inoculated and control leaves were 4.82 and 14.00 µmoles/m^{-2}-sec, respectively, when the internal carbon dioxide concentration was approximately 310 µl/l for each (Table 6). At other internal carbon dioxide concentrations, the assimilation rates for the inoculated leaf were only 21 to 54% and 23 to 40% of those for the control leaf, in the presence of Hoagland's solution and PEG solutions, respectively (Figure 6, Table 6). At all internal carbon dioxide concentrations, the total assimilation rates of the

control leaf under stress were an average of 95% as large as the total assimilation rates for the same control leaf in Hoagland's solution, while that of the inoculated leaf under stress was only an average of 82% as large as the total assimilation rates for the same leaf in Hoagland's solution (Figure 6, Table 6). The total carbon dioxide assimilation rates give no clue as to whether the decrease in the rates for the inoculated leaf was a result of increased respiration or a decrease in photosynthetic carbon fixation. However, any decrease in photosynthetic carbon fixation seen here could result from changes in the inoculated leaf, which was more chlorotic than the control leaf.

The data from this experiment must be interpreted with caution because the experiment was not repeated. However, conductance and diffusive resistance are both measurements of stomatal aperture. In this experiment, stomatal apertures which can fluctuate over time were monitored continuously. The conductance data are in agreement with the diffusive resistance data. Nevertheless, the data concerning the relationship between the carbon assimilation and internal carbon dioxide concentration must be evaluated with caution. The differences in rates of stomatal closure observed after exposure to moderate water stress must also be evaluated with caution.

Effects of Fungal Toxin, Graminin A, on the Stomatal Activity of Wheat

In both experiments performed, diffusive resistances were reduced in the first leaves of water-stressed seedlings that were poainted with 5 µg/ml, 25 µg/ml, 100 µg/ml and 500 µg/ml one half hour after treatment (Figure 7). Mean diffusive resistances were significantly lower for leaves treated with 5 and 100 µg/ml one-half hour after treatment at the 0.05 level, using a T test than that of the control leaves (Table 7). Mean diffusive resistances for leaves treated with the remaining concentrations were significantly lower at the 0.1 level, than that of the control leaves. The mean diffusive resistances for all treatment concentrations were still lower than that of the control 1.5 hours after treatment, but these differences were not significant except for the 5 µg/ml concentration.

Only the mean values for the 5 µg/ml concentration were significantly smaller at both 0.5 and 1.5 hours than the mean diffusive resistance for those leaves before treatment (0 hours). The author interprets this to mean that the plants had not been exposed to the PEG solutions long enough, and as the Graminin A was lowering the diffusive resistances, the PEG solution was still raising them. The fact that these two forces operate against each other may explain the lack of statistical differences between the means of the majority of the concentrations for leaves before and after treatment. This also may explain why only two treatment concentrations (5 and 100 µg/ml) produced means that were

FIGURE 7. Effect of Graminin A on adaxial stomatal diffusive resistances (sec/cm) of water-stressed leaves. Water stress was induced by placing roots of the seedlings in a PEG solution of -6 bars water potential. Several concentrations (5 µg/ml, 25 µg/ml, 100 µg/ml, 500 µg/ml) of Graminin A were painted onto the adaxial leaf surfaces.

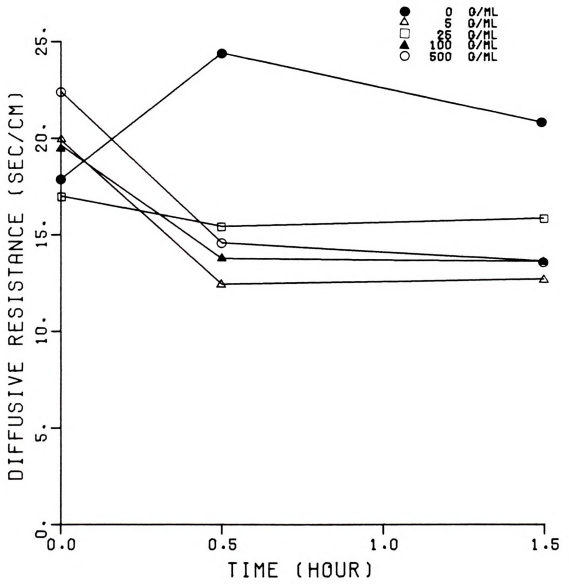
0 Q/ML
5 Q/ML
25 Q/ML
100 Q/ML
500 Q/ML
DIFFUSIVE RESISTANCE (SEC/CM)
0.
5.
10.
15.
20.
25.
0.0
0.5
1.0
1.5
TIME (HOUR)

Figure 7

TABLE 7. Effect of Graminin A on adaxial stomatal diffusive resistances of water-stressed leaves. Water stress was induced by placing roots of the seedlings in a PEG solution of -6 bars water potential. Several concentrations (5 µg/ml, 25 µg/ml, 100 µg/ml, 500 µg/ml) of Graminin A were painted onto the adaxial leaf surfaces. Mean values (sec/cm) of the first leaves are given.

Concentration of Graminin A	Time (hours) 0	0.5	1.5
0 µg/ml	17.89	24.4[a,b,f,g,h]	20.8
5 µg/ml	19.9[c,d,e]	12.42[b,c,d]	12.68[e]
25 µg/ml	17.84	15.42[f]	15.84
100 µg/ml	19.64	13.76[a,g]	16.60
500 µg/ml	22.41	14.57[h]	13.61

[a,b,c] Diffusive resistances that are statistically different at the 0.05 level using a T test.

[d,e,f,g,h] Diffusive resistances that are statistically different at the 0.1 level using a T test.

statistically different from that of the control at the 0.05 level. Nevertheless, enough statistical differences were produced to conclude that Graminin A opens stomates in the leaves of water-stressed plants.

DISCUSSION

Increased stomatal opening in infected plants has been reported for only a few diseases (2,3,17); in most cases, the water potential-stomatal aperture relationship was not examined. Therefore, the data showing that C. gramineum causes increased stomatal aperature before the appearance of chlorotic stripes was of interest. Variation in the diffusive resistances among both the inoculated and control leaves was great. In spite of the relatively large variation, significant differences between the mean diffusive resistances of the inoculated and control plants were found soon after inoculation (Table 3, Figure 1). Next, a system was designed which accentuated the differences in diffusive resistances between healthy and diseased plants. Stomatal closure of healthy plants is known to occur under moisture stress (37). Therefore, roots of hydroponically-grown inoculated and control plants were placed in PEG solutions of varying water potentials.

Several different techniques have been used to study the changes in stomatal apertures or solute potentials caused by various disease, by examining inoculated and control plants at the same water potentials. Water has been withheld from plants in order to study the differences in the water potential-stomatal

aperture relationship between water-stressed (low water potentials) rusted and control bean plants (16). Fully turgid inoculated and control plants were used by Ayres (2). Samples of leaf tissue from inoculated and control plants have been floated on nutrient solutions (15) or water (13) for adjustment of leaf water potential. We believe this is the first attempt to manipulate the water potentials of plants, by producing uniform leaf water stress quickly and easily, using solutions of polyethylene glycol in conjunction with hydroponically-grown plants.

Several related findings resulted from the study: (a) It was found that the stomates of diseased plants open wider and longer than those of healthy plants (Tables 3, 4, 6; Figures 1, 2, 5, 6) especially under conditions of water stress. (b) The stomatal responses in diseased plants were sluggish, in response to water stress the stomated of diseased plants did not operate as quickly and efficiently as did the stomates of healthy plants (Tables 4, 6; Figures 2, 5, 6). (c) The relationship between water potentials and diffusive resistances in diseased plants was altered from that of healthy plants, implying the involvement of a diffusable toxin in the disease process (1,4,12,42) (Table 5, Figure 3). (d) And finally, the differences in stomatal aperture were not caused by differences in leaf water status (Table 5, Figure 3). This implies that blockage of the vascular system by the fungus or its metabolites does not play a role in early pathogenesis. Blockage appears to cause leaf stripes. Blockage probably is involved in the later stages of the disease (48).

Many diseases have been reported to affect transpiration rates and water loss from plants through changes in stomatal aperture (2,3,15,16,19,27), damage to the cuticle of the plant (3,15,16), or other mechanisms (2,17). The water potential-stomatal aperture relationship has been altered, as reported here for Cephalosporium stripe disease, in only a few diseases. However, in many experiments examining the effects of various diseases, the appropriate measurements to test for changes in that relationship were never made (2,3, 10,15,17,27). For example, infection of tobacco by downy mildew (Peronospora tabacini) caused an increase in transpiration during the night, before sporulation occurred (10). After sporulation, the transpiration rate decreased. Since stomatal aperture was not measured, the relative contribution to the transpiration rates from stomatal aperture, the fungus itself, and damage to the cuticle cannot be determined. More than likely, however, water lost from the fungus itself increased the dark transpiration before sporulation, and stomatal closure reduced it following sporulation.

Two diseases that decrease transpiration during pathogenesis are powdery mildew of wheat and bean rust. During the first six hours following inoculation with Erysiphe graminis f. sp. tritici, the stomates of inoculated wheat plants were more closed than those of control wheat plants. The transpiration rate in the light of the diseased plants was significantly less than that of the control plants (27). Water potential measurements were not taken, and it is not known whether or not the water potential-stomatal apertrue relationship was altered. Beans infected with Uromyces phaseoli,

had reduced transpiration rates and reduced stomatal apertures in the light at four to six days after inoculation. In this instance the ability of stomates to respond to changes in the environment apparently was altered (15).

The experiments on bean rust made use of discs from rsut-infected and control bean leaves (five days after inoculation) which were floated on nutrient solutions. The leaf discs from both control and inoculated leaves should have been equilibrated to the same water potential, that of the nutrient solution. The fact that the stomates of the inoculated discs opened more slowly and did not open as much as those of the control discs indicated that the water potential-stomatal aperture relationship was probably altered. However, discs from both healthy and inoculated leaves closed in the dark immediately. Once sporulation occurred, the transpiration rate of diseased leaves was greater than that of healthy leaves and did not respond to changes in illumination. Nevertheless, the stomates of diseased leaves were more closed than those of control leaves. The increased transpiration rate was caused by the ruptured cuticle. With bean rust, alteration of the relationship between water potential and stomatal aperture resulted in more closed stomates. This is in contrast with wheat plants infected with C. graminеum, which have stomates more open than those of controls. The effects of Uromyces phaseoli occurred only within 5 mm of the pathogen (15); this also differs from the case with Cephalosporium stripe, where the pathogen is sequestered at some distance from the affected stomata.

Abnormal stomatal opening has been reported in several other diseases. Peas infected with Erysiphe pisi, the causal organism of powdery mildew, had increased transpiration and stomatal opening in both the light and in the dark by two days after inoculation (3). From the second to the seventh days after inoculation, transpiration from diseased plants was less and the stomatal apertures were smaller in the light and greater in the dark than those of control leaves. In the diseased plants, however, the stomatal opening in the light declined more than did the transpiration rate. On the seventh day, all stomatal movements ceased, and the stomates of diseased plants remained partially open both in the light and in the dark. When the fungus was removed from diseased leaves, transpiration in the light increased. Therefore, it was thought that very little water was released from the fungus, and that the fungus on the surface of the leaf decreased transpiration by changing the boundary layer resistance of theleaf. The authors also proposed that the mechanical forces of the mycelium growing across the leaf and invading the cells might affect stomatal opening. The significance of the transitory stomatal opening in the light is therefore not clear because of the confounding effects of the fungus on the leaf and because the relationship between water potential and stomatal aperture was not examined.

Phytopthora infestans was reported to affect stomates in potato plants. The stomatal apertures of a narrow band (approximately 6 mm wide) around necrotic lesions were reported to be greater than the apertures of stomates in non-infected areas of the leaf

(17). Infection-induced opening of stomates occurred in the light and in the dark. However, the fungal mycelium was found to extend 1-2 mm beyond the outer edge of this zone of increased stomatal aperture. An increase in the osmotic values of the guard cells in the affected area was reported, and is not surprising. Perhaps the guard cells absorbed the solutes leaked from the cells in the necrotic lesion (5). Also, stomates exposed to the decrease in back pressure resulting from the death of the cells in the adjacent lesion would be expected to open. However, non-diseased controls were not examined and measurements of water potential and stomatal aperture were not taken; thus, no conclusion can be made on whether or not the increase in stomatal aperture was caused by an alternation in the water potential-stomatal aperture relationship.

Rhyncosporium secalis, causal organism of barley blotch, cause changes in stomatal action. The stomates of barley leaves near the areas colonized by Rhynochosporium secalis were more open than those of control leaves (2). This phenomenon occured only after the pathogen invaded the epidermal and mesophyll cells of the host. The solute potentials of inoculated and control plants at full turgor were measured as an attempt to determine whether the water relations of the diseased plant had been altered. When the cell membranes beomce more leaky, the cell loses solutes, and the solute potential, expressed with a negative sign, increases to a less negative value. In the diseased leaves, the solute potentials for both epidermal and guard cells were less negative than the potentials for control leaves. This indicated that the permeability of

the membranes of both the epidermal and guard cells had changed. Since the solute potentials dropped more in the epidermal cells than in the guard cells, the back pressure on the guard cells was reduced and the stomates opened.

The phytotoxin fusicoccin, from Fusicoccum amygdali (the cause of bud canker of almond) is known to affect stomates. The toxin and the fungus also induce wilitng and necrotic lesions at a distance from the site of application or infection (18). Fusicoccin was shown to open the stomates of almond (45), bean (19), Commelina communis (38), and other plant species (18). When fusicoccin was added to Commelina communis, the subsidiary cells failed to take up a vital stain (38). A dead cell leaks solutes and the solute potential drops. Potassium uptake by guard cells was greatly increased in bean (Phaseolus vulgaris) when treated with fusicoccin (41,43). These changes created a situation similar to that for barley blotch; the difference between the solute potentials of epidermal cells and guard cells was increased so that the back pressure on the guard cells was reduced and the stomates opened.

The relationship between water potential and stomatal aperture should be altered in the bud canker disease of almond caused by Fusicoccum amygdali, but this has never been examined. The only available data show that fusicoccin produced by the fungus alters the water potental-stomatal relationship. Water potential and diffusive resistance dropped dramatically in dogwood leaves treated with fusicoccin, while both parameters remained fairly constant in the controls (18,44).

Under stress, the stomates of plants infected by C. gramineum are also more open than those of the control plants. For stomates to open, the water potentials of guard cells must be greater than that of epidermal cells. In this respect the situation in leaves infected by C. gramineum and R. secalis is similar to that of leaves treated with fusicoccin.

Cephalosporium stripe disease differs from barley blotch in that the pathogen remains distant from the epidermis, instead of invading the epidermal cells as R. secalis does. A toxin similar to fusicoccin that opens stomates appears to be involved in the formation of yellow leaf stripes on wheat infected with C. gramineum. But since the diffusive resistance of C. gramineum-infected leaves increased with increasing water stress, as did the diffusive resistances of control leaves (Table 5, Figure 3), the release of epidermal back pressure did not cause the diseased leaves to exhibit lower diffusive resistances relative to that of the controls.

Several diseases have been shown to affect stomatal action, causing them to be more open and more closed than normal. However, as far as the author knows, Cephalosporium stripe of wheat is the first example of a disease that induces stomatal opening and for which there are data on the relationship between water potentials and stomatal aperatures.

One requirement used in evaluating the significance of toxins is that the toxin reproduce the pertinent effects of infection (32). In this study, the stomates of wheat seedlings infected with Cephalosporium gramineum were more open than those of healthy

seedlings under water stress. The water potential-diffusive resistance studies reported here imply the existence of a toxin. It should be possible to use stomatal effects as an aid in evaluating toxins proposed to be factors in development of this disease.

Graminin A, a toxin produced by C. gramineum in culture, is reported to cause extensive chlorosis in wheat cuttings treated with 25 µg/ml in three days (23). Non-host species such as beans and tomato are not affected. The leaves of the cuttings turn brown and wilt in five days. Vascular browning, a characteristic symptom of the disease also developed in culms of the cuttings.

In this study, Graminin A has been shown to significantly decrease adaxial stomatal diffusive resistances when painted on leaves. The fact that this fungal toxin causes stomatal opening as well as chlorosis and vascular browning implies that the toxin is involved in the early stages of the disease process.

SUMMARY

The stomates of wheat plants infected by Cephalosporium gramineum were open wider and responded more slowly than those of healthy plants, before the appearance of chlorotic stripes. This phenomenon was especially evident under conditions of water stress. PEG solutions were used to induce water stress quickly and uniformly and to manipulate leaf water potential. Diffusive resistances for both inoculated and control leaves increased as water stress was increased, indicating that the release of epidermal back pressure did not cause the significantly lower diffusive resistances of the inoculated leaves.

Extensive work has been done to determine whether vascular blockage or a diffusible toxin causes particular symptoms in several diseases, including Cephalosporium stripe. Examination of the relationship between leaf water potentials and stomatal apertures indicated that the differences in stomatal activity were not caused by differences in leaf water status, implying the involvement of a diffusible toxin. Blockage of the vascular system by the fungus or its metabolites probably does not play a significant role in early pathogenesis.

Graminin A, a toxin produced by C. gramineum, caused abnormal stomatal opening in seedlings subjected to water stress. This implies that Graminin A has a role in disease development.

Graminin A, a toxin produced by C. gramineum, caused abnormal stomatal opening in seedlings subjected to water stress. This implies that Graminin A has a role in disease development.

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